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# Application of wavelet and Fuorier transforms as powerful alternatives for derivative spectrophotometry in analysis of binary mixtures: A comparative study

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ABSTRACT

Two signal processing methods, namely, Continuous Wavelet Transform (CWT) and the second was Discrete Fourier Transform (DFT) were introduced as alternatives to the classical Derivative Spectrophotometry (DS) in analysis of binary mixtures. To show the advantages of these methods, a comparative study was performed on a binary mixture of Naltrexone (NTX) and Bupropion (BUP). The methods were compared by analyzing laboratory prepared mixtures of the two drugs. By comparing performance of the three methods, it was proved that CWT and DFT methods are more efficient and advantageous in analysis of mixtures with overlapped spectra than DS. The three signal processing methods were adopted for the quantification of NTX and BUP in pure and tablet forms. The adopted methods were validated according to the ICH guideline where accuracy, precision and specificity were found to be within appropriate limits.



## 1. Introduction

Interference in spectroscopy can be eliminated by many techniques such as signal processing. In analytical chemistry, signal processing can be used to sharpen peaks, resolve spectra, carry out quantitative analysis, and remove background interference. Derivative Spectrophotometry (DS) is one of the most widely used signal processing techniques [1,2]. In UV–VIS spectroscopy, derivative takes a great part in overlapped spectra resolution. The zero crossing method was successfully applied for the quantitative determination of different mixtures [3–5].

Several disadvantages are observed due to the application of usual numerical derivative to the absorption spectra. Among these are peak intensity reduction obtained with higher order derivatives and the requirement of smooth function and the scaling factor which may deform the peak shape from the original one upon derivative calculation. These drawbacks necessitated introduction of other signal processing techniques as replacements to derivative calculation such as Continuous Wavelet Transform (CWT) [6,7] and Discrete Fourier Transform [8]. CWT involves the decomposition of a signal function (e.g., UV–VIS spectrum) into simpler, fixed building units at diverse scales and positions [9,10]. As convolution using combined trigonometric Fourier functions adjusts all types of interferences, application of these functions to absorbance data would lead to removal of interferences from other components and remove background noise [8,11]. CWT and DFT were used on raw spectra and on ratio spectra for analysis of different binary and ternary mixtures [12–14].

Naltrexone hydrochloride (NTX), (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one Fig. 1a [15], is used to suppress pro-opiomelanocortin (POMC) inhibition and so augmenting greater effect of POMC activation [16]. Bupropion hydrochloride (BUP), 2-(tert-Butylamino)-1-(3-chlorophenyl)propan-1-one Fig. 1b [15], is a dopamine and norepinephrine reuptake inhibitor also stimulates POMC neurons in the hypothalamus, resulting in decrease of appetite and increased energy output [16]. In 2014, FDA has approved a new combination for the treatment of obesity and controlling body weight containing NTX and BUP. This combination can be used efficiently in management of obesity by targeting CNS pathways that affect food intake.

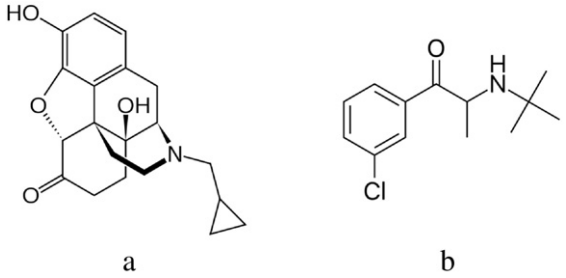


**Fig. 1.** Structural formulae for a) Naltrexone b) Bupropion.

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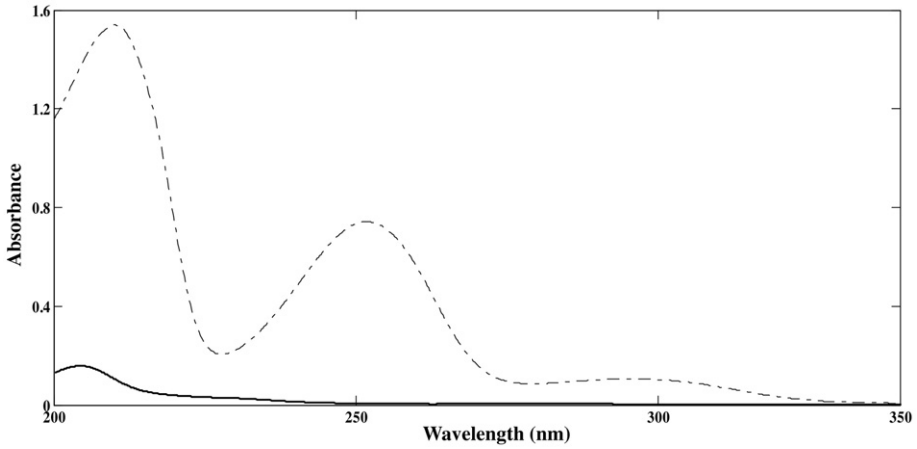


**Fig. 2.** Zero order absorption spectrum of 1.6 μg/ml NTX (___) and 18 μg/ml BUP (- - -) using distilled water as blank.

Several techniques were used for the quantification of BUP including spectrophotometry [17–19] and chromatography either in dosage form [20,21] or in biological fluids [22–25]. Also, it was determined together with its metabolites [26–34] or its degradation products [35]. NTX was determined using chromatographic techniques either liquid chromatography [36–40] or gas chromatography [41–43]. Also, different electrochemical techniques [44–49] and spectroscopic techniques [50–53] were applied for the quantification of NTX. Few methods were adopted for the simultaneous quantification of both drugs including HPLC [54, 55] and colorimetry [56].

The main goal of this work was to present alternative methods to the classical DS such as CWT and DFT and a comparative study was established to show the differences between these methods on the analysis of BUP and NTX in binary mixture. Another goal was to develop simple, sensitive, accurate and economic analytical methods for the quantification of BUP and NTX either in their pure or tablet forms.

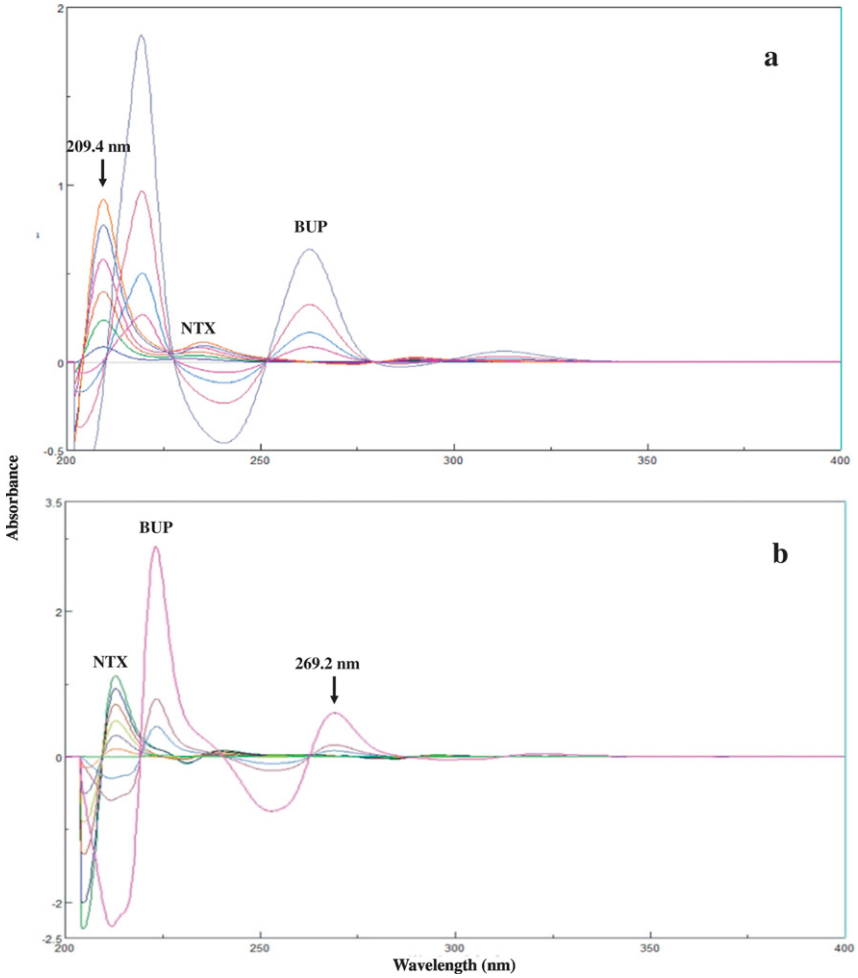


**Fig. 3.** 1st order (a) and 2nd order (b) derivative spectra of NTX and BUP using DS method, showing zero-crossing points for NTX (a) and BUP (b) determination.

## 2. Experimental

### 2.1. Chemicals and Materials

Pure BUP and NTX were purchased from Cayman chemical company, Ann Arbor, United States of America (USA); their purity was certified to be 99.9%. Distilled water from "Aquatron" Automotive Water Still A 4000 [bibby Sterillin Ltd., Staffordshire-UK).

### 2.2. Instrumentation

Double beam spectrophotometer (JASCO, Japan) with 1-cm pathlength matched quartz cuvettes. It is connected to IBM compatible computer with HP 680 inkjet printer (Hewlett Packard, USA).

### 2.3. Software

CWT calculations were applied using Matlab® version 7.12, (R2011a), while DFT calculations were done by Microsoft® Excel 2010. The calculations were adopted using a Dual CPU, 1.47 GHz, 2.00 GB RAM operated by Microsoft Windows 7™.

### 2.4. Pharmaceutical Formulations

Contrave® extended-release tablets (NDC 51267-890-99) labeled to contain 8 mg NTX and 90 mg BUP. It was manufactured by Orexigen Therapeutics Inc., La Jolla, California, USA.

### 2.5. Standard Solutions

NTX stock standard solution (0.25 mg/mL) was prepared by accurate weighing and transferring of 25 mg pure NTX into 100-mL volumetric flask. The drug was dissolved by aid of a vortex mixer in 50 mL distilled water then the volume was completed using the same solvent. BUP stock standard solution (1 mg/mL) was prepared by the same manner but using 50 mg pure BUP into 50-mL volumetric flask.

Working standard solutions for the studied drugs were prepared by diluting 5 mL of, NTX stock standard solution (0.25 mg/mL) or BUP stock standard solution (1 mg/mL) into two separate 50-mL volumetric flasks using distilled water as a diluting solvent to get working standard solutions of concentrations 25 μg/mL and 100 μg/mL for NTX and BUP, respectively.

### 2.6. Procedures

#### 2.6.1. Spectral Characteristics of NTX and BUP

Zero-order ($D_0$) absorption spectra of 1.6 μg/mL NTX and 18 μg/mL BUP were recorded using distilled water as a blank over the range of 200–350 nm.

#### 2.6.2. Calibration Curves Construction

Definite volumes of NTX working standard solution (25 μg/mL) equivalent to 25–275 μg were accurately and separately transferred into a series of 25-mL volumetric flasks and the volume of each flask was completed to the mark with distilled water. On the other hand, aliquots of BUP working standard solution (100 μg/mL) equivalent to 30–250 μg were accurately and separately transferred into a series of 10-mL

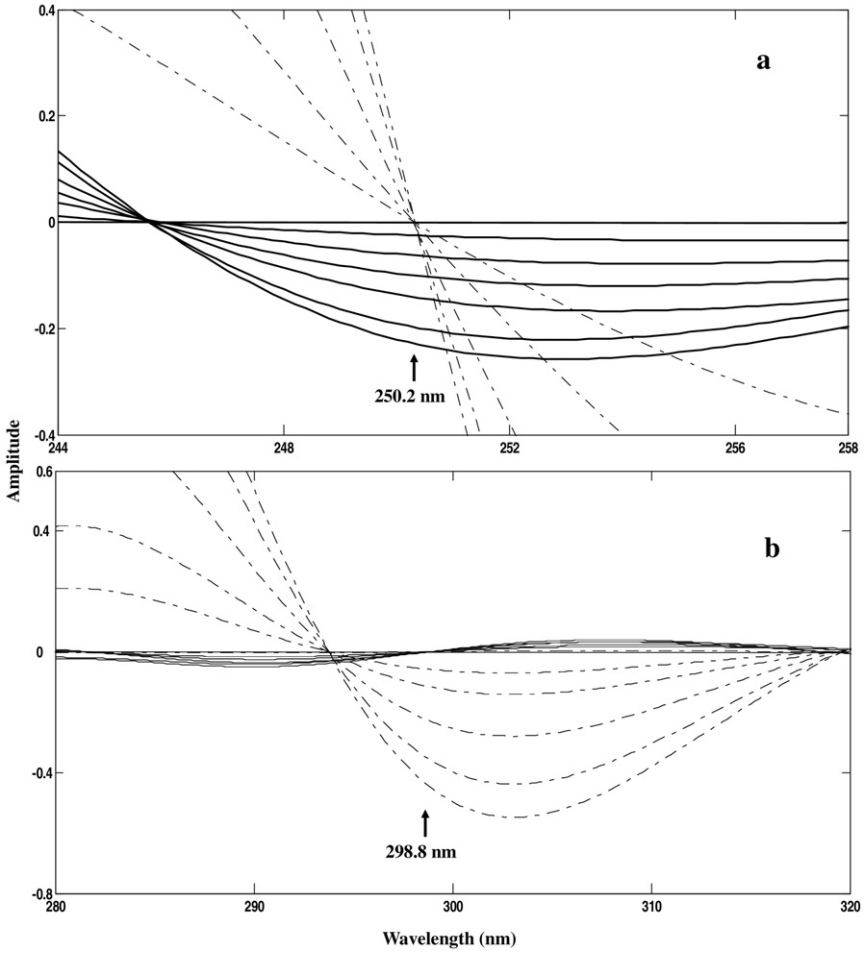


**Fig. 4.** CWT of 1–11 μg/ml NTX (——) and 3–25 μg/ml BUP (- - -) showing zero-crossing points for NTX (a) and BUP (b) determination.

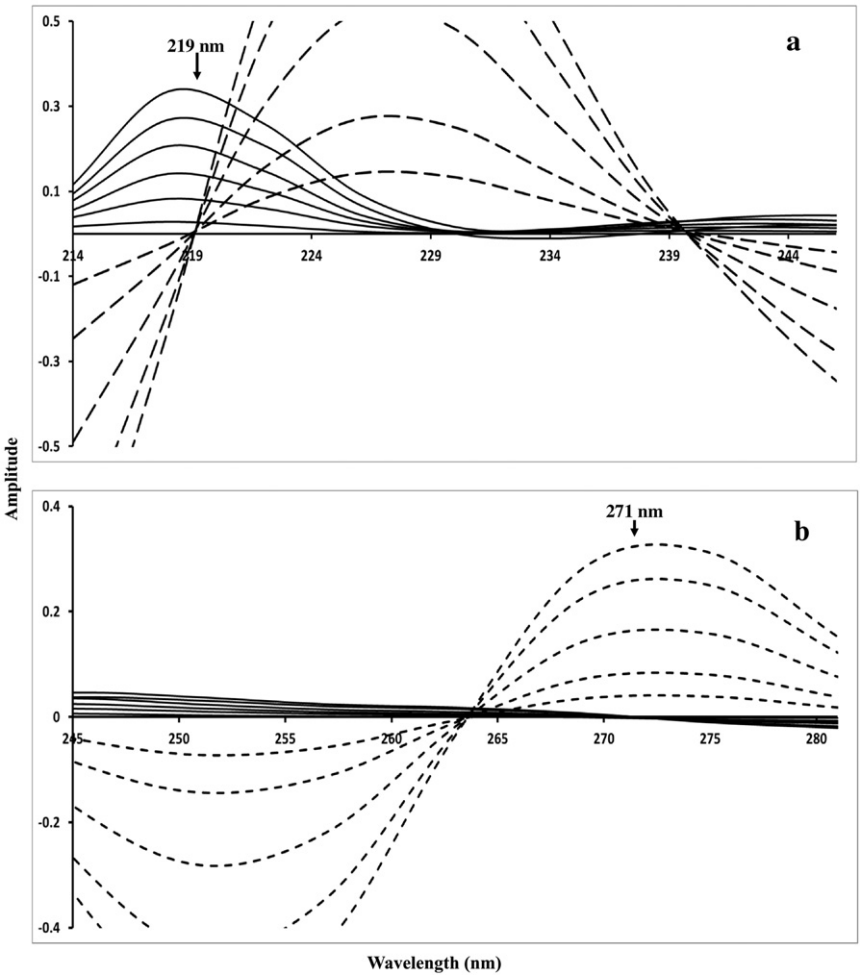


**Fig. 5.** DFT of 1–11 μg/ml NTX (——) and 3–25 μg/ml BUP (- - -) showing zero-crossing points for NTX (a) and BUP (b) determination.

volumetric flasks and the volume of each flask was completed to the mark with distilled water. The absorption spectra were recorded using distilled water as blank.

*2.6.2.1. DS.* NTX was determined by measuring the $^{1}$D-peak heights at 209.4 nm, the first derivative spectra were calculated using $\Delta\lambda = 8$ nm and scaling factor = 10. BUP was quantified by measuring the $^{2}$D-peak heights at 269.2 nm, the second derivative spectra were calculated with $\Delta\lambda = 16$ nm and scaling factor = 100.

*2.6.2.2. CWT.* The CWT of NTX and BUP spectra was calculated with daubechies wavelet family (db-5) and scale = 100. The amplitude of CWT of NTX was measured at 250.2 nm, while the amplitude of CWT of BUP was measured at 298.8 nm. Calibration graphs were plotted between the peak amplitude at 250.2 and 298.8 nm and the corresponding concentrations of NTX and BUP, respectively.

*2.6.2.3. DFT.* The Fourier Transform of NTX was obtained using eight points, $T' = [\mathrm{Cos}\,X + \mathrm{Cos}(X + 45)]$, combined Fourier function, the different coefficients were calculated from the respective absorbance values each 4 nm interval in the range 200–260 nm. The Fourier Transform of BUP was obtained using six points, $T' = [\mathrm{Cos}\,X + \mathrm{Cos}(X + 60)]$, combined Fourier function, the different coefficients were calculated from the respective absorbance values each 6 nm interval in the range 230–296 nm. The coefficients of NTX at $\lambda_m = 219.0$ nm and of BUP at $\lambda_m = 271.0$ nm were plotted against the corresponding concentrations and calibration curves were constructed.

**Table 1**
Determination of NTX and BUP in laboratory prepared mixtures by the proposed methods.

| Concentration (μg/mL) | | DS | | CWT | | DFT | |
|---|---|---|---|---|---|---|---|
| | | Recovery %[a] | | | | | |
| NTX | BUP | NTX | BUP | NTX | BUP | NTX | BUP |
| 1.6 | 18 | 99.51 | 101.54 | 100.12 | 98.80 | 102.19 | 98.44 |
| 1.6 | 22 | 100.49 | 100.74 | 99.53 | 97.94 | 101.00 | 98.25 |
| 3.0 | 18 | 100.98 | 100.11 | 101.20 | 100.11 | 99.06 | 100.31 |
| Mean ± SD | | 100.33 ± 0.75 | 100.80 ± 0.72 | 100.28 ± 0.85 | 98.95 ± 1.09 | 100.75 ± 1.58 | 99.00 ± 1.14 |

[a] Average of three determinations.

**Table 2**
Determination of NTX and BUP in Contrave® tablets by the proposed spectrophotometric methods.

| Drug | DS | CWT | DFT |
|---|---|---|---|
| | Recovery % ± SD[a] | | |
| NTX (1.6 μg/mL) | 99.49 ± 1.12 | 99.12 ± 0.91 | 100.21 ± 0.78 |
| BUP (18 μg/mL) | 100.89 ± 1.32 | 100.19 ± 0.83 | 98.92 ± 0.96 |

[a] Average of three determinations.

*2.6.3. Application of the Proposed Methods for the Determination of NTX and BUP in Laboratory-Prepared Mixtures*

Aliquots of NTX and BUP were taken from their standard working solutions into a single series of 10-mL measuring flasks resulting in mixtures with diverse ratios of the two drugs. Concentrations of NTX and BUP were computed using the previous procedures.

*2.6.4. Application of CWT, DFT and DS Methods for the Determination of NTX and BUP in Contrave® Tablets*

Ten Contrave® extended release tablets were weighed to get the average weight of a tablet then crushed, finely powdered and mixed well. Tablet powder equivalent to 8 mg NTX/90 mg BUP was transferred to a beaker of 250 mL capacity then; a suitable volume of distilled water (40 mL) was added and stirred for about 20 min. Filtration was carried out into 100-mL volumetric flask. Washing of the residue was done using about 20 mL distilled water (twice). The volume was completed to the mark with distilled water and mixed well, then 1 mL of the prepared solution was transferred to a 50-mL volumetric flask and the volume was completed to the mark with distilled water and mixed well. The spectra were recorded and the concentration of the two drugs were calculated using the procedures described under construction of calibration curves.

## 3. Results and Discussion

The absorption spectra of NTX and BUP show overlapped spectra in the region 200–250 nm (Fig. 2). This overlap hinders the determination of NTX in presence of BUP, especially with the low amount of NTX in the dosage form compared to BUP. Also BUP can't be determined directly in presence of NTX.

The classical derivative calculation (DS) method was developed for determination of the two drugs using different orders. A zero crossing point for NTX was detected in the first derivative at 209.4 nm, while for BUP no suitable points were observed in the first derivative spectra. This necessitated the use of higher order derivatives to get a zero crossing point for BUP. By calculating the second order spectra, a zero crossing point for BUP was observed at 269.2 nm. The parameters that influence the performance of derivative calculations such as Δλ and scaling factor were optimized. Δλ of 4 and 8 nm were used for the first and second order derivatives, respectively, and produced noisy spectra. So the best results were obtained using Δλ = 8 nm and scaling factor = 10 for the first order, while for the second order best performance was obtained with Δλ = 16 nm and scaling factor = 100. Good linearity was obtained in a range of 1.0–11.0 μg/mL and 3.0–25.0 μg/mL for NTX and BUP, respectively (Fig. 3).

The aim of this work was to search for other signal processing methods that provide zero crossing points with more advantages than DS. CWT and DFT were developed for determination of the two drugs.

For analysis of the binary mixture using CWT algorithm, several wavelet families were investigated in different orders such as Haar, Symlets (*sym*), Daubechies (db), Morlet (morl), Gaussian (gaus), Meyer (meyr), Coiflets (coif) and Mexican hat (mexh). The only factor that must be adjusted after choosing the suitable wavelet is the scaling parameter ($a$). The best wavelet, regarding zero crossing points, signal to noise ratio and the recovery in laboratory prepared mixtures, was db-5 with scaling ($a$) = 100. The CWT of BUP showed several zero crossing points at 228.4, 250.2, 272 and 293.4 nm, while those of NTX showed points at 223.6, 244.6, 268.2, 284.2 and 298.8 nm. For determination of NTX, the amplitude at zero crossing 250.2 nm provided best recovery in the laboratory prepared mixtures, while for BUP, the zero crossing at 298.8 nm gave the best results. Satisfactory linearity was attained in the range of 1.0–11.0 μg/mL and 3.0–25.0 μg/mL for NTX and BUP, respectively (Fig. 4).

For analysis using DFT, different parameters related to computation of the Fourier coefficients were adjusted. The trigonometric functions (Sin and Cos) were tested, and the Cos was preferred as it participated more to the spectra over the wavelength range chosen. The combined trigonometric Fourier function coefficients, $t'_{ij}$, were computed from absorbance values using 6 points, $T' = [\cos x + \cos(x+60)]$, and 8 points, $T' = [\cos x + \cos(x+45)]$, and at different intervals (2, 4, 6 and 8 nm). For NTX, the best DFT was obtained using $T' = [\cos x + \cos(x+45)]$ at 4 nm interval, while for BUP, the optimum DFT was that resulting from $T' = [\cos x + \cos(x+60)]$ at 6 nm interval.

The convoluted spectra of BUP showed zero crossing points at 219 and 288 nm, while the convoluted spectra of NTX showed zero crossing points at 271 and 293 nm. The points at 219 and 271 nm showed the best results concerning linearity and recovery in laboratory prepared mixtures for NTX and BUP, respectively. Suitable linearity was observed

**Table 3**
Statistical comparison for the results obtained by the adopted methods and the reported HPLC method [54] for the analysis of NTX and BUP in pure powder.

| Value | CWT | | DFT | | DS | | Reported method[a] | |
|---|---|---|---|---|---|---|---|---|
| | NTX | BUP | NTX | BUP | NTX | BUP | NTX | BUP |
| Mean | 100.57 | 100.92 | 100.58 | 100.82 | 100.75 | 100.92 | 101.48 | 102.17 |
| RSD% | 0.524 | 0.519 | 0.679 | 0.502 | 0.930 | 1.017 | 0.974 | 1.193 |
| n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Variance | 0.278 | 0.275 | 0.466 | 0.256 | 0.876 | 1.053 | 0.977 | 1.485 |
| Student's *t*-test[b] (2.3) | 1.817 | 2.101 | 1.679 | 2.295 | 1.202 | 1.758 | – | – |
| F value[b] (9.6) | 3.512 | 5.405 | 2.097 | 5.802 | 1.115 | 1.410 | – | – |

[a] RP-HPLC using $C_{18}$ and phosphate buffer and acetonitrile (60:40, *v*/v) as mobile phase. The UV detection was carried out at 224 nm at a flow rate of 1.0 mL/min.
[b] The values in the parenthesis are the corresponding theoretical values of t and F at $P = 0.05$.

**Table 4**
Validation sheet of CWT and DFT methods for the simultaneous determination of the binary mixture.

| Parameter | CWT | | DFT | | DS | |
|---|---|---|---|---|---|---|
| | NTX | BUP | NTX | BUP | NTX | BUP |
| Accuracy[a] | 99.02 | 100.19 | 101.46 | 98.89 | 100.75 | 99.29 |
| Precision | | | | | | |
| Repeatability[b] | 0.83 | 0.69 | 0.97 | 0.89 | 0.61 | 0.74 |
| Intermediate precision[c] | 1.01 | 0.88 | 1.21 | 1.14 | 1.01 | 0.78 |
| Linearity | | | | | | |
| Slope | −0.1930 | −0.0761 | 0.308 | 0.1300 | 0.0845 | 0.0238 |
| Intercept | 0.0116 | −0.0048 | −0.0117 | 0.0013 | −0.0133 | 0.0063 |
| Correlation coefficient (r) | 0.9996 | 0.9999 | 0.9992 | 0.9999 | 0.9991 | 0.9999 |
| Range (μg/ml) | 1–11 | 3–25 | 1–11 | 3–25 | 1–11 | 3–25 |

[a] The accuracy ($n = 3$), mean recovery of three concentrations (3, 5, 7 μg/mL) for NTX and (3.125, 6.25, 12.5 μg/mL) for BUP.
[b] The intraday ($n = 3$), RSD of three concentrations (3, 5, 7 μg/mL) for NTX and (3.125, 6.25, 12.5 μg/mL) for BUP repeated three times within day.
[c] The interday ($n = 3$), RSD of three concentrations (3, 5, 7 μg/mL) for NTX and (3.125, 6.25, 12.5 μg/mL) for BUP repeated three times in three days.

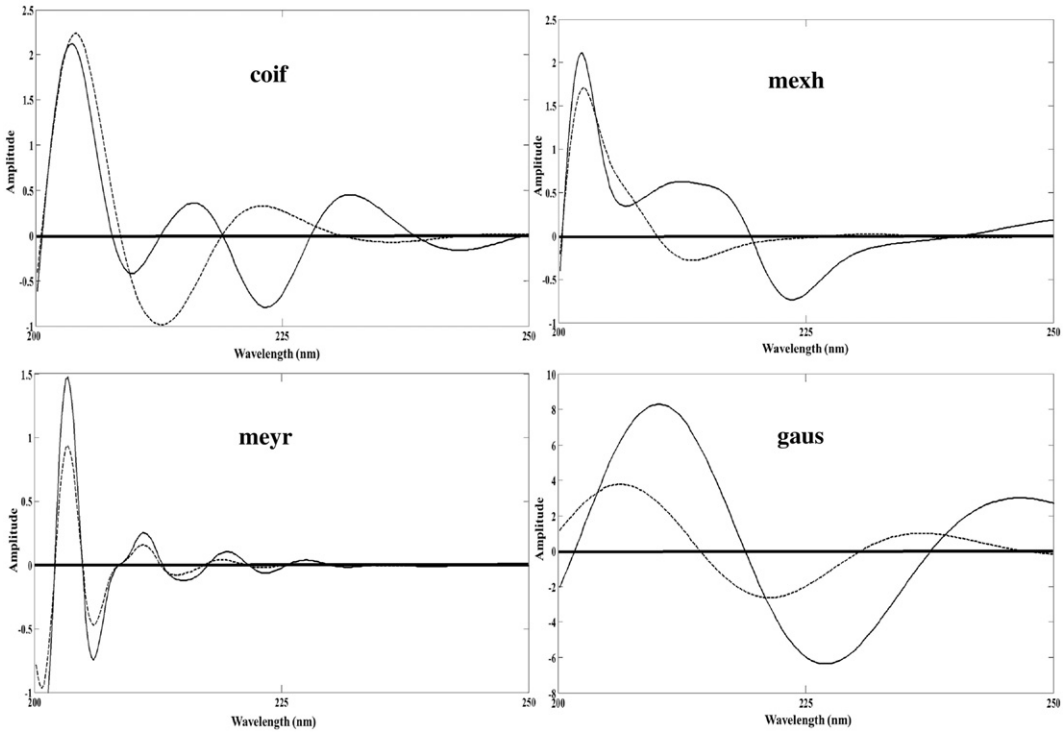


**Fig. 6.** Different first derivative spectra of NTX (- - -) and BUP (——) using four different CWT families.

in the concentration range of 1.0–11.0 μg/mL and 3.0–25.0 μg/mL for NTX and BUP, respectively (Fig. 5).

The selectivity of the proposed methods was evaluated by analysis of binary mixtures containing different proportions of the drugs (Table 1). The proposed methods were utilized for the determination of NTX and BUP in Contrave® tablets (Table 2). The results acquired by the proposed methods for analysis of the drugs in pure powder were statistically compared to those of the reported HPLC method [54]. No significant difference was observed between the developed methods and the reported one as presented in Table 3. The methods were validated according to ICH guideline [57] and the parameters were collected in Table 4.

During the trials to reach a zero crossing points for determination of NTX and BUP, CWT and DFT showed several shapes of processed spectra which increased the possibility of zero crossing (Figs. 6 and 7). This flexibility arise from using various families in CWT and different Fourier functions in DFT. With many possible zero crossing points, one can choose the point in regard of the best sensitivity and selectivity. As a result of this advantage we could determine the two drugs simultaneously using db family in CWT, in contrast to DS where simultaneous determination was not possible.

One of CWT advantages is signal amplification compared to other signal processing methods [58]. This may be noticed in the zero crossing point selected for NTX determination which was 250.2 nm. Although NTX shows very low absorptivity at 250.2 nm (Fig. 2), CWT could show good linearity and determine NTX at this wavelength. DS and DFT could only show linearity and determine NTX in the range 200–220 nm at which NTX has high absorptivity. This can be attributed to the lack of signal amplification in contrast to CWT. This advantage offers CWT additional flexibility for choosing zero crossing points than other methods.

## 4. Conclusion

From the previous results, we come to a conclusion that the suggested methods are accurate, selective, precise and simple over the specified linearity ranges and can be applied for analysis of NTX and

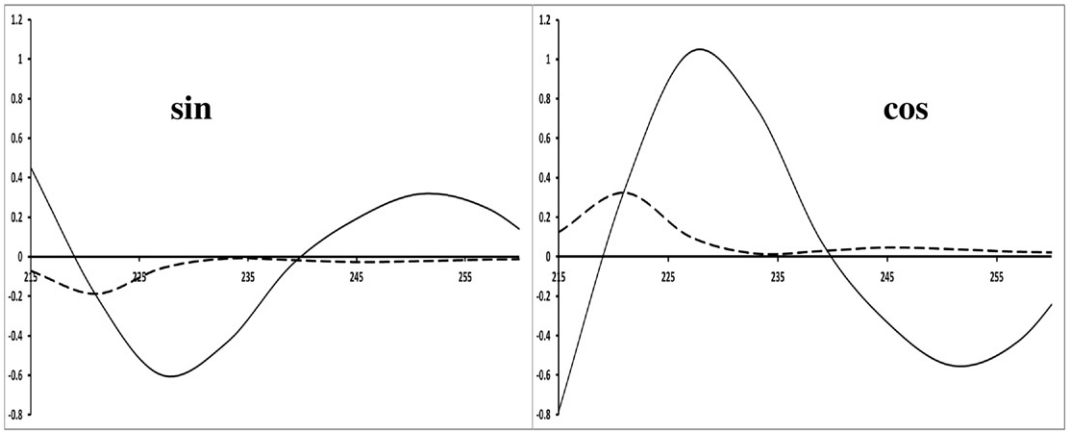


**Fig. 7.** DFT of NTX (- - -) and BUP (——) using cos and sin functions.

BUP in their mixture. The methods are simple and suitable for laboratories lacking liquid chromatographic instruments. The CWT and DFT methods showed advantages over the classical derivative methods, and both can be considered as powerful alternatives. CWT offer better probabilities for finding zero crossing points than DFT, by more number of wavelet families and signal amplification.

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